USE OF MOLECULAR TOOLS IN PABRA

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Centro Internacional de Agricultura Tropical
International Center for Tropical Agriculture
Consultative Group on International Agricultural Research

Eco-Efficient Agriculture for the Poor

MAJOR PRODUCTION CONSTRAINTS OF BEANS IN AFRICA (WORTMANN, ET AL. 1998)

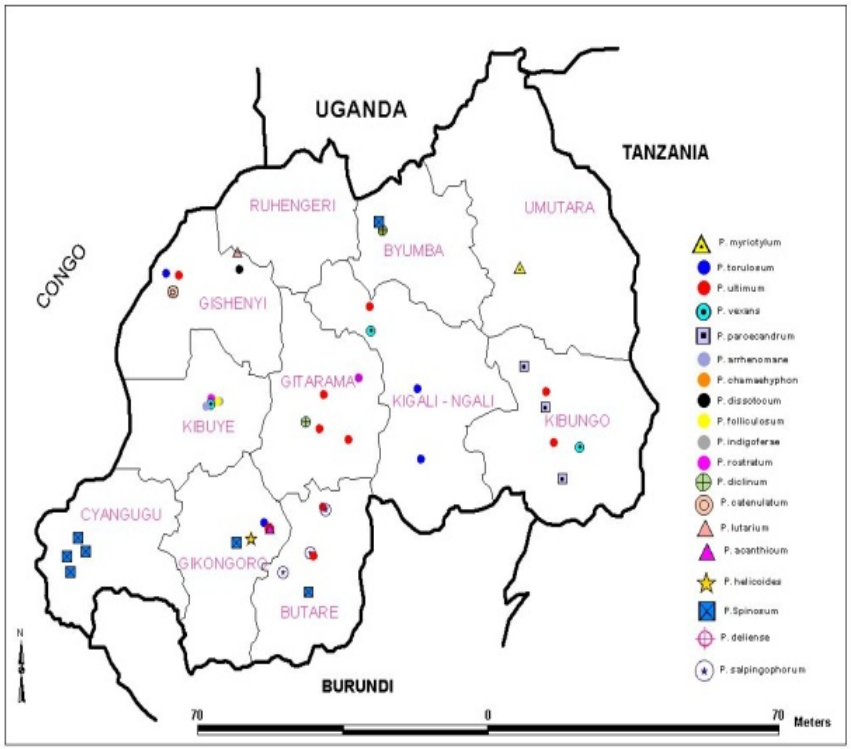
Type	Constraint	Yield loss t (p.a.)
Abiotic	Drought	396,000
	N deficiency	389,900
	P deficiency	355,900
	Al/Mn toxicity and exch. bases	383,900
Biotic	Angular leaf spot	384,200
	Anthracnose	328,000
	Bean stem maggot	297,100
	Root rots	221,100
	CBB	220,400

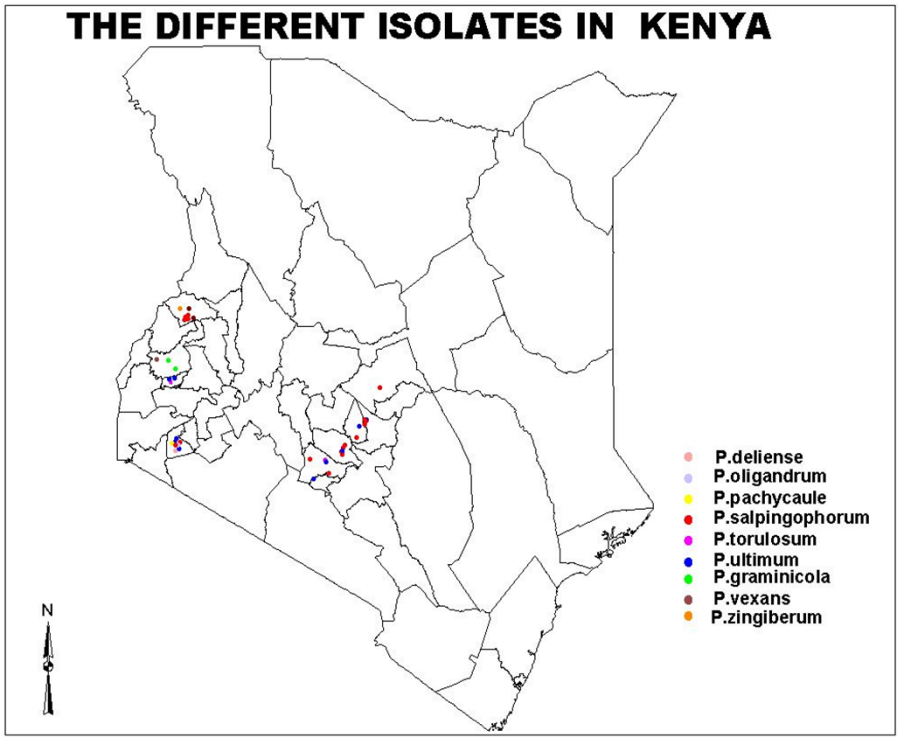
HISTORY OF BIOTECHNOLOGY

- Within the last 100 years the world has seen the rise of genetics as a scientific discipline (1900s),
- The finding of DNA as the hereditary material (1944),
- The elucidation of the double helix structure of the DNA molecule (1953),
- The cracking of the genetic code (1966),
- The ability to isolate genes (1973),
- The application of DNA recombinant techniques (from 1980 onwards).
- There are several applications of Biotechnology in crop improvement

APPLICATION OF BIOTECHNOLOGY IN BEAN IMPROVEMENT

- Initial focus on ALS and Pythium root rot
- Molecular diagnostic tools to characterize and study the spatial distribution pathogens/races





MARKER ASSISTED SELECTION

- **Marker Assisted Selection: Integration** of marker-aided breeding with conventional approaches is aimed at enhancing **the efficiency and effectiveness of common bean breeding in Africa**

- MAS refers to the use of DNA markers that are tightly-linked to target loci as a substitute for or to assist phenotypic screening.

- By determining the allele of a DNA marker, plants that possess particular genes or quantitative trait loci (QTLs) may be identified based on their genotype rather than their phenotype

BENEFITS OF MARKERS ASSISTED SELECTION

- Speed breeding cycle by selecting at seedling stage
- Increase precision and effectiveness (markers linked to genes of interest)
- Efficient pyramiding genes of desirable traits
- Screening without the target constraint (e.g. pathogens or races) to identify sources of resistance or make selections

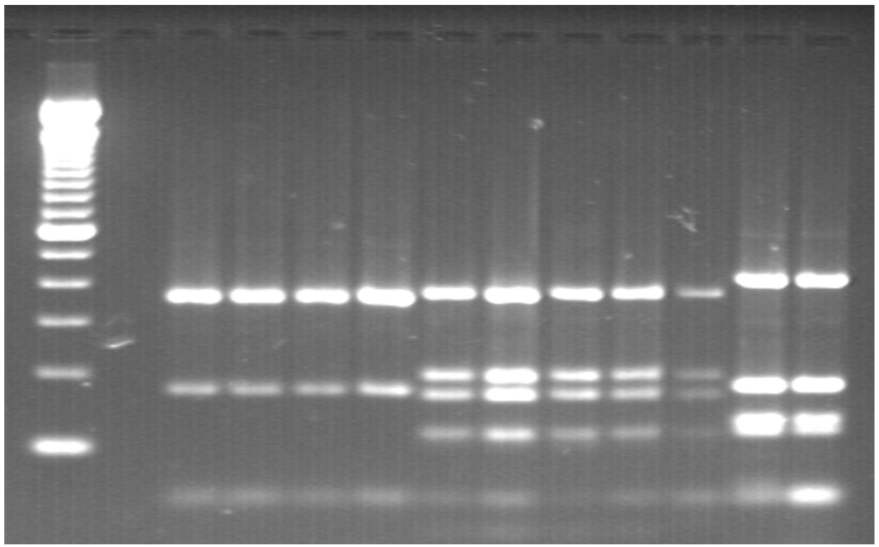


Fig 2: Banding pattern using oomycetes specific primer

MARKER ASSISTED BREEDING-PABRA

ROCKFELLER FOUNDATION

- Which traits are important and amenable?
- Development of Markers
- Capacity development
- Application in breeding

AFRICAN BEAN BREEDING CONSORTIUM (ABC) FUNDED BY THE KIRKHOUSE TRUST

Objectives

- Introduce the capability of marker-assisted selection (MAS) into African bean breeding program
- infrastructure & training in MAS
- Determine which traits are important and amenable
- **Developing Varieties Tolerant to Two or More Major Biotic Stresses in Africa (ALS, ANT, BCMV & BCMNV, CBB, and Pythium Root Rot)**
- Project Development and planning of activities

PROJECTS CURRENTLY FUNDED

1. **Application of molecular marker assisted selection in developing common bean varieties with improved multiple disease resistance to *Pythium* root rot, bean common mosaic necrotic virus and anthracnose in Uganda**

2. **Use of marker-assisted selection (MAS) to improve selection efficiency in breeding for resistance to major diseases of common bean (*Phaseolus vulgaris* L.) in Tanzania.** CBB, ALS, BCMV/BCMNV

3. **Strengthening Capacity for Marker Assisted Breeding for Common Bean in eastern Africa (Kenya)** -Anthracnose, ALS, Root rot and BCMV

DISEASE TARGETED FOR MAS

Common Bacterial Blight

Bean Common Mosaic Virus

Angular Leaf Spot

Pythium Root rot

Bean Common Mosaic Necrotic Virus

Anthracnose

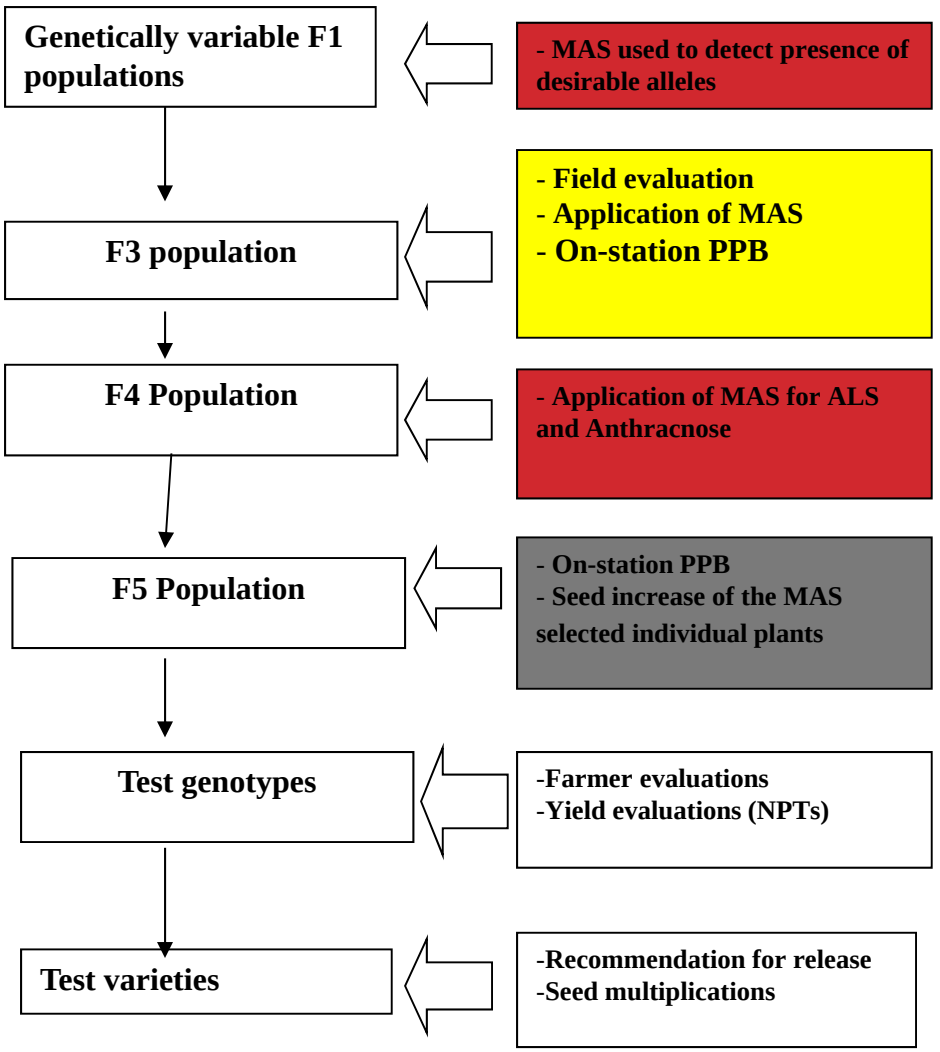
MAS IN BEAN IMPROVEMENT

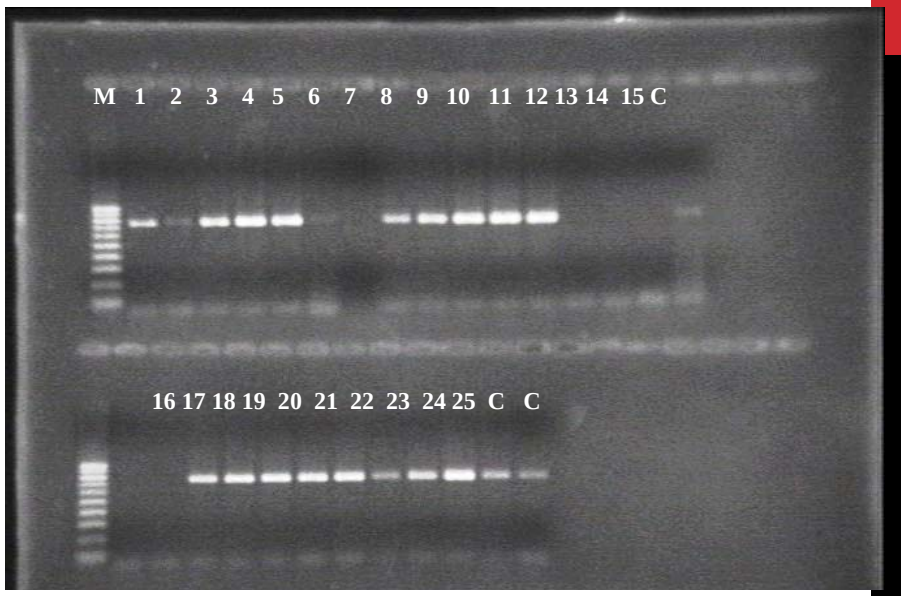
- √ Identification of sources of resistance
- √ Establishment of the nature of resistance and the genes involved
- √ Identification, validation and development of protocols for markers associated with resistant genes
  - √ *Initial focus was on Angular Leaf spot and Pythium root rot*
- √ Introgression of genes and application of markers in selection (E.G., *I* and *bc3* gene for BCMV/BCMNV)
- √ Development of Multiple disease resistance breeding parents (Angular Leaf spot, Pythium root rot, Anthracnose, BCMV and BCMNV)

MARKERS BEING UTILISED

Trait	Markers	Source
ALS	$OPE4_{709}$	Mex 54 (Mahuku et al,2004)
	$PF9_{260}$	G10474 and G10909
Pythium root rot	PYAA19	RWR 719 (Buruchara et al
	PYBA08	RWR 719
Anthracnose	SAB -3	G2333
	SAB 13	G2333
	SAB 14	G2333, AB 136
BCMV	ROC 11	Various
BCMNV	SW 13	Various
CBB	$SAP6_{820}$	Miklas et 2000; Deidre et al 2007

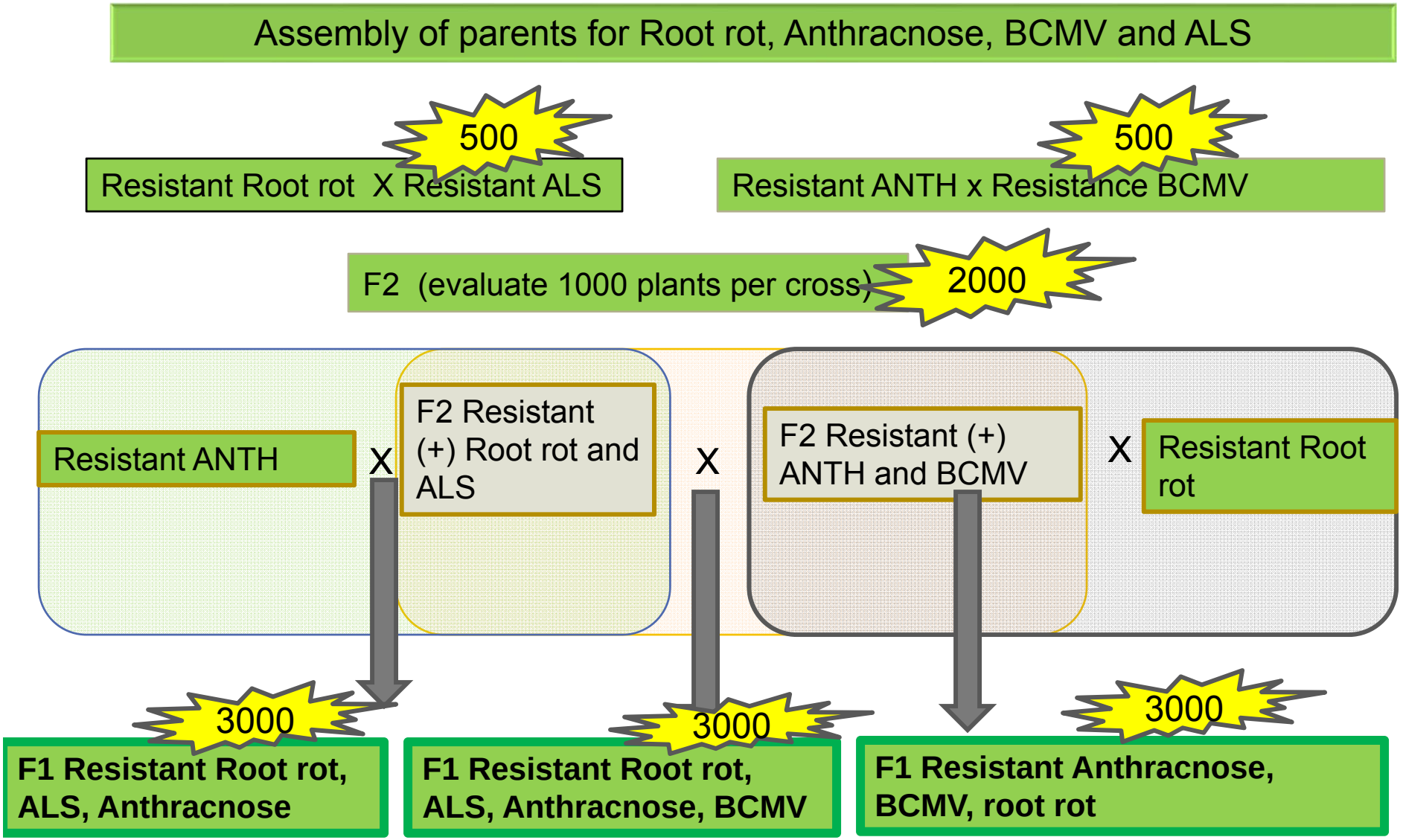
EXAMPLE 1: F_1 to F_5 recombinant inbred lines developed from multiple crosses combining ALS, Pythium and anthracnose





SCAR marker and resistance gene genotype for RWR 719 gene

Example 2: Development of multiple disease resistance breeding parents



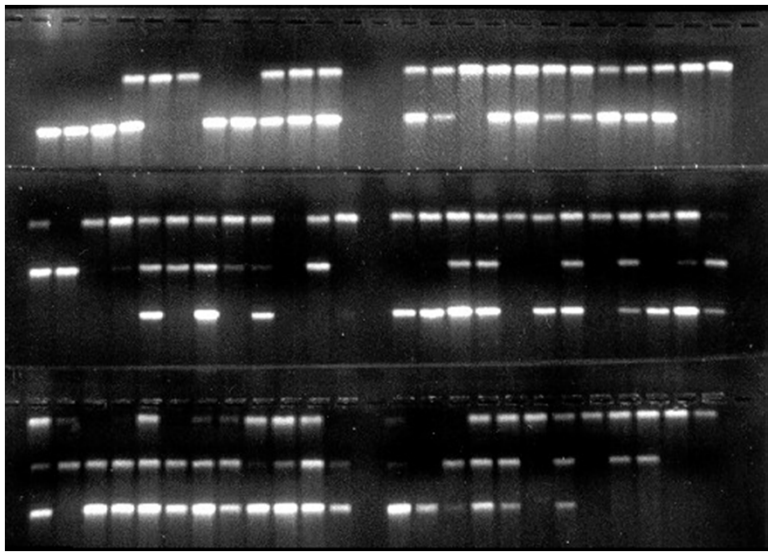
Combining Resistance To Biotic And Abiotic Stresses

Pyramiding: eg. ((VAX6 x BRB191) x (G17722 x AND277))

CBB BCMV Drought ALS

Marker assisted selection:

SW13)



BCMV (ROC11,

CBB (SAP6, SU91)

Bruchids (Arc)

DISEASES AND PESTS NOT BEING ADDRESSED

Web blight

Haloblight

Ascochyta Leaf spot

Bean Rust

PESTS NOT BEING ADDRESSED

BSM

Ootheca

Pod borers

Pod suckers

Aphids

Bruchids

Whiteflies

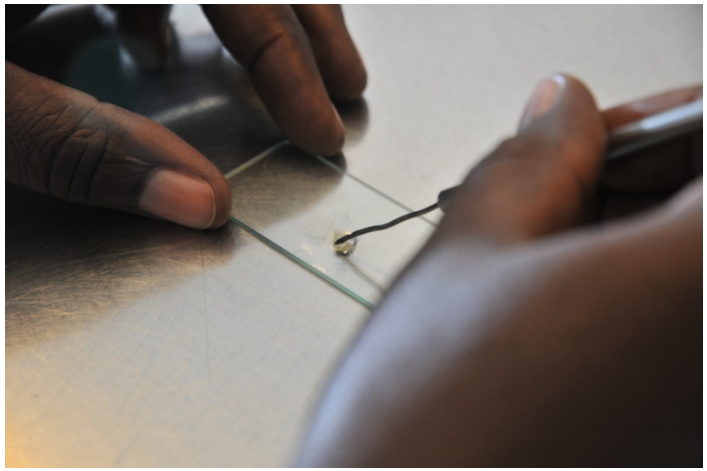
TRAINING IN DISEASE PHENOTYPING AND MAS-KAWANDA

- **Attachments**
- **Visits to NARs stations**
- **Post graduate student Research**
- **Training courses**

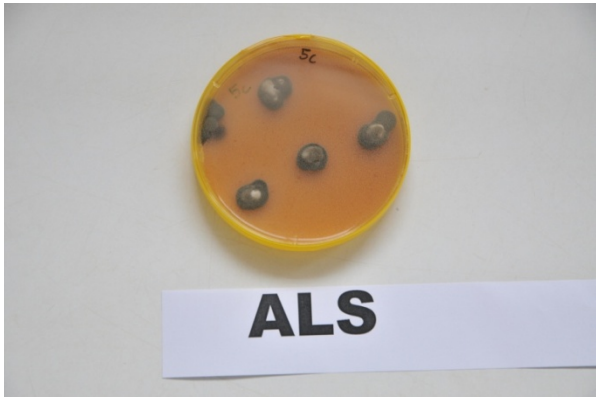
READING, DISCUSSIONS AND INTERACTION

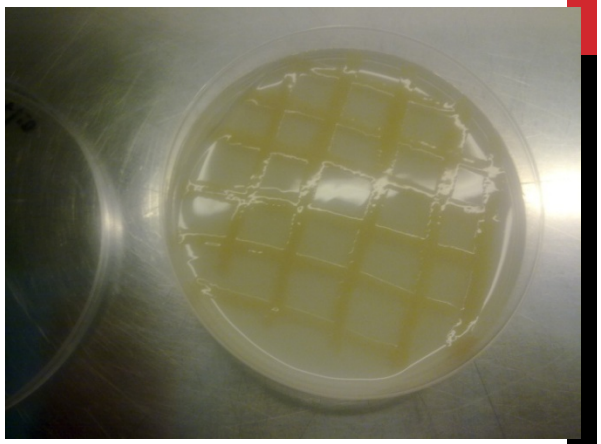
DISEASE SCOUTING: IDENTIFICATION AND SAMPLE COLLECTION

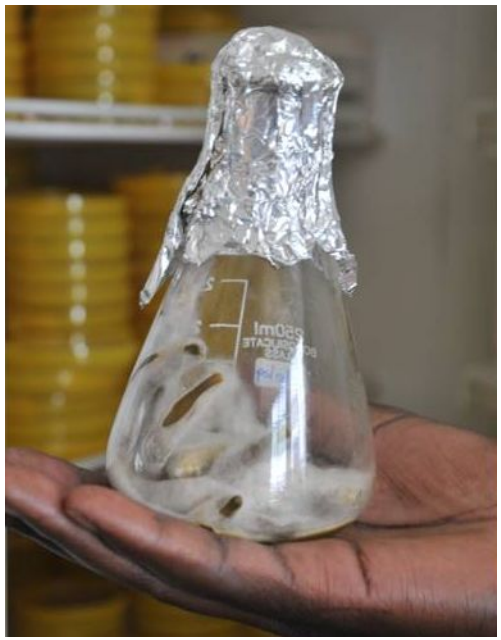
ISOLATION



PATHOGEN CULTURING







INOCULUM PREPARATION

INOCULATION

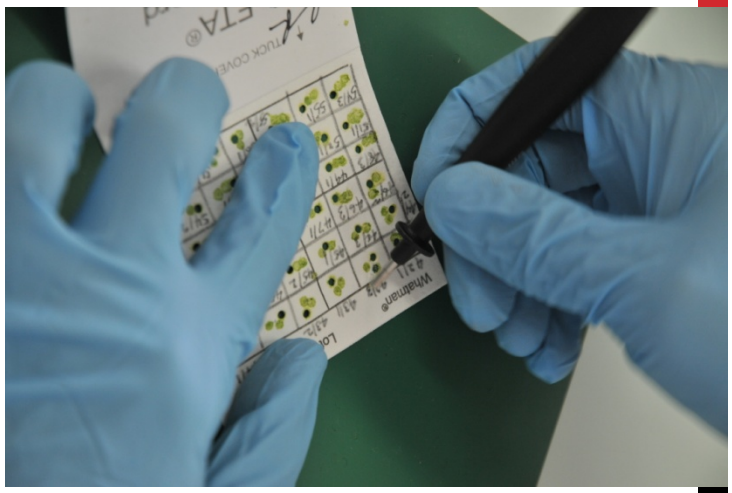






DISEASE EVALUATION

MARKER ASSISTED SELECTION



OTHERS

Culture storage

Crossing

- Experimental design,
- Data analysis and interpretation
- Common bean breeding methods
- Simple seed storage techniques

LESSONS LEARNT

Potential exists to apply MAS for certain traits

Capacity is limiting

- Skills
- For more partners to apply in breeding
- Increase throughput

Need for more new and robust markers and protocols

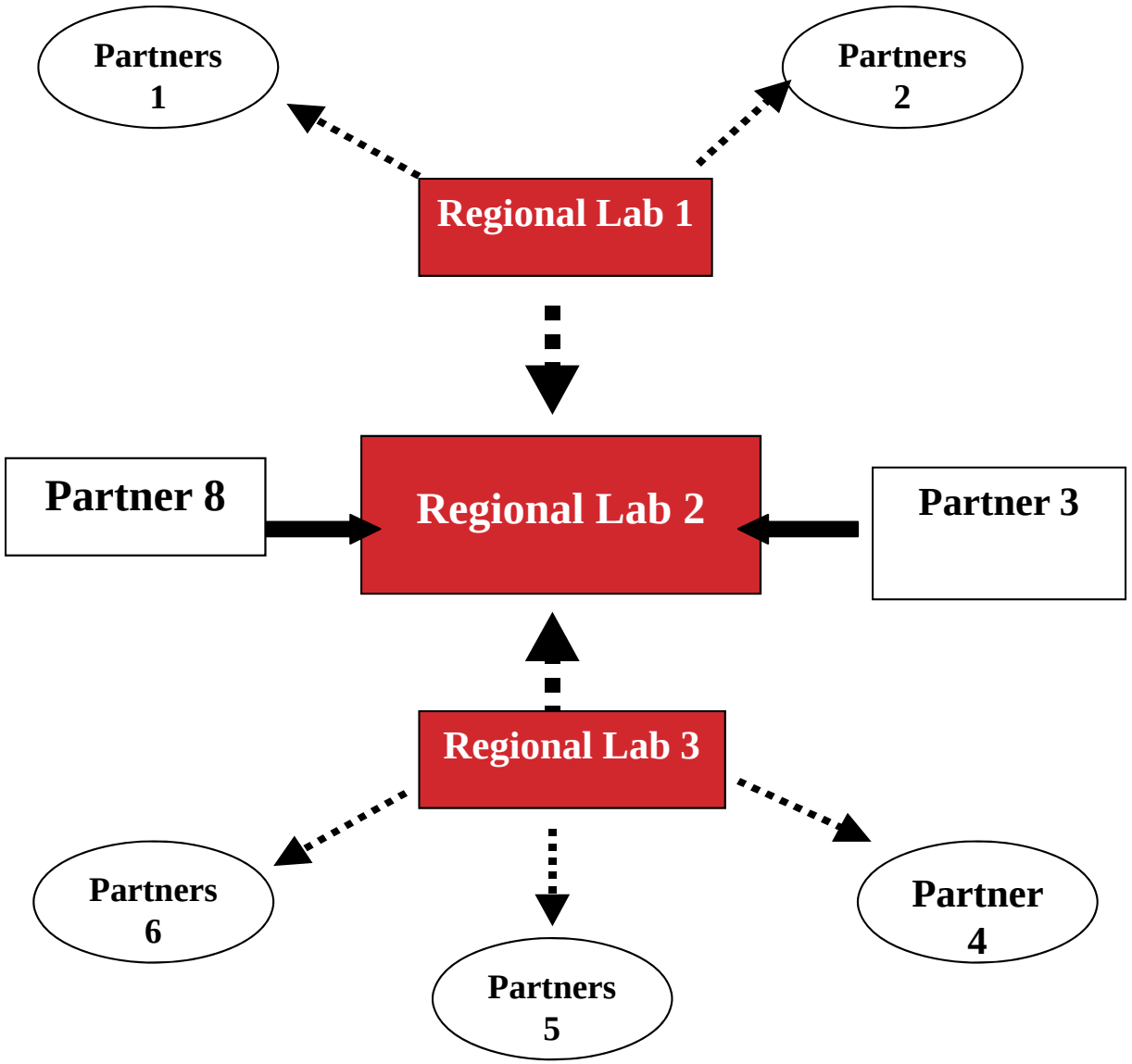
Integration into breeding program: examples:

- When is MAS applied relative to phenotypic selection for other traits?

PROBABLE OBSTACLES FOR THE ADOPTION OF MAS

- Prohibitive cost
  - Equipment and consumables required to establish and maintain a marker lab
  - Large initial cost in the development of markers
- Low reliability of markers to determine phenotype
  - (‘*thoroughness’ of the primary QTL mapping study; even QTLs that are detected with high LOD scores and explain a large proportion of the phenotype may be affected by sampling bias and may not be useful for MAS*)
- Effect of a QTL may depend on the genetic background
  - (*important to test the QTL effects and the reliability of markers (i.e. QTL/marker validation) before MAS is undertake*n.
- Level of integration between molecular geneticists and plant breeders (and scientists from other disciplines) may not be adequate to ensure that markers are effectively applied for line development.

Proposed Marker Assisted Selection “Partnership”



THANK YOU!!